RESPIRATION OF A COLORLESS FLAGELLATE, ASTASIA KLEBSII

HERMAN VON DACH[1]

(From the Zoology Department, Ohio State University,[2] and the Marine Biological Laboratory, Woods Hole)

Introduction

There is a growing literature on protozoan respiration recently reviewed by Jahn (1941), but the sum total of research done in this field is still quite small and not comparable to the work done on respiration of bacteria, yeast and metazoan tissues. In the belief that a detailed study of the respiratory characteristics of a flagellate would have some value from the standpoint of comparative physiology, the present work was undertaken. This paper deals with the effects of various factors on the respiration of *Astasia klebsii.*

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Materials and Methods

Astasia klebsii, the species used in this investigation, is a free-living, colorless, plantlike flagellate. Its nutrition is saprozoic. Food reserves of paramylum, a starch-like polysaccharide which does not respond to the usual iodine test for starch, are stored as small granules in the cell. The same strain employed in a previous investigation (Von Dach, 1940) was used.

Pure (bacteria-free) clone cultures of *Astasia klebsii* were grown at 27° C. in one liter Erlenmeyer flasks containing 500 cc. of the following organic medium:

[1] Present address: Physiology Department, Georgetown University Medical School, Washington, D. C.

[2] Muellhaupt Scholar, 1939–1940.

KNO_3	0.5 gm.
KH_2PO_4	1.5 gm.
$MgSO_4 \cdot 7H_2O$	0.1 gm.
NaCl	0.1 gm.
$CaCl_2$	0.01 gm.
$FeCl_3$	trace
Sodium acetate	2.0 gm.
Bacto-tryptone	5.0 gm.
Distilled water	1000 cc.

(Adjusted to pH 5.8 with N/1 NaOH or N/1 HCl)

After incubation the cells were concentrated by centrifuging, washed three times in inorganic buffer by centrifuging, and suspended in buffer. The buffers used were potassium phosphate mixtures (M/20 K_2HPO_4–KH_2PO_4) containing the following added ingredients per liter: KNO_3 0.09 gm., $MgSO_4 \cdot 7H_2O$ 0.09 gm., NaCl 0.09 gm., $CaCl_2$ trace, $FeCl_3 \cdot 6H_2O$ trace. The proportions of K_2HPO_4 and KH_2PO_4 were varied according to the pH desired, after Sorenson's tables (Peters and Van Slyke, 1932, p. 816). The pH of the buffers was checked by a Hellige colorimetric comparator or a glass electrode. It was found that the cells survived longer and with less deterioration in these buffers than they did in M/20 phosphate alone.

Respiration of the cell suspensions was measured by the Warburg direct method. The standard procedures of this method, as described by Dixon (1934), were employed.

All the experiments were conducted at 25.2° C. ($\pm$ 0.05°). The vessels were shaken continuously at from 70 to 90 cycles per minute, through an arc of from 4.5 to 7.5 cm.; these rates were found to be wholly adequate.

A portion of each cell suspension was fixed in 10 per cent formalin for determining cell concentration. Cell counts were made by means of a Sedgwick-Rafter counting cell and a Whipple ocular micrometer. Usually between four and eight million cells per vessel were used.

At the end of an experiment the cell suspensions were removed from the respirometer vessels for the following tests: (1) pH determination; (2) microscopic examination for possible cell injury and bacterial contamination; (3) in some cases, cell counts.

In testing for cell injury and death a modification of the method of Devereux and Tanner (1927) was used. Two or three drops of cell suspension were mixed with a drop of 0.5 per cent erythrosin on a slide and examined. Cells which did not stain and which displayed movement, whether swimming or protoplasmic contraction, were regarded as uninjured. Cells which did not move at all and which nearly always

became stained were regarded as irreversibly injured or dead. Repeated tests have shown the validity of this criterion for *Astasia*.

No increase in cell number was observed to occur during an experiment. Except where otherwise noted, the number of injured astasias remained negligible, and the pH of the suspensions remained constant within 0.2 or 0.3 unit, throughout the experiments.

The number of bacteria present in the cell suspensions was negligible in all cases. While slight bacterial contamination undoubtedly occurred as soon as the pure cultures were exposed to the air, the process of quickly washing the cells and suspending them in inorganic buffer kept bacterial growth at a minimum. These experiments, then, may be regarded as essentially bacteria-free.

The gas space of the vessels contained air in all cases. Most of the experiments were conducted at pH 5.8; this pH had previously been found most favorable for growth (Von Dach, 1940). Most of the experiments were of two or three hours' duration. Results were calculated as cubic millimeters of oxygen consumed per hour per million cells, or as cubic millimeters of carbon dioxide produced per hour per million cells. In nearly all cases, the values for duplicate respirometers did not differ from each other by more than 7 per cent.

Results

I. *Respiration in Inorganic Medium*

The growth of *Astasia klebsii* cultures was found to be similar to that of yeast and bacteria. In newly-inoculated cultures there occurred in succession: (1) the "logarithmic growth phase" (which usually lasted about 120 hours), during which growth proceeded at a constant maximal rate until a cell concentration of about 300,000 cells per cc. was reached; (2) the "phase of negative growth acceleration," during which growth continued briefly at a decreasing rate; (3) the "stationary phase," during which the cell population remained fairly constant at approximately 650,000 cells per cc. for at least five weeks, with no appreciable amount of cell division or cell death.

In these experiments it was not convenient to regulate the initial cell concentrations of the culture; since duration of the logarithmic phase must increase as the initial cell concentration is decreased, it would not be enough to merely state the ages of the various cultures here. Classification of cultures as to phase of growth was based on a study of their growth curves.

Figure 1 shows the relation of rate of oxygen consumption in inorganic medium to time. Experiments on several cultures in the loga-

rithmic phase and several in the stationary phase are represented. These data show the following general relationships:

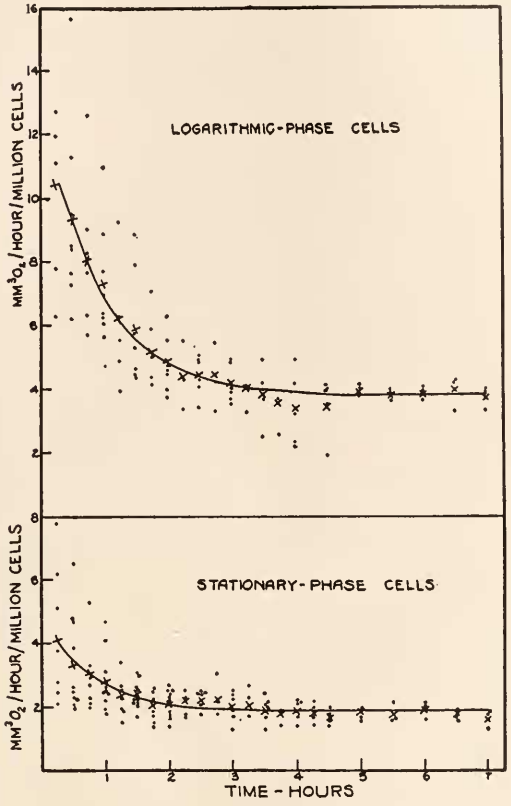

FIG. 1. Change in rate of respiration in inorganic buffer with time (pH 5.8). Dots, values from separate experiments; crosses, average values. (Logarithmic-phase cells from cultures 86 to 162 hours old; stationary-phase cells from cultures 188 to 504 hours old.)

For both old and young cells, an early period of declining respiration was followed by a more or less constant period. In logarithmic-phase cultures (i.e. "young" cells) there was an average oxygen consumption of 10 cu. mm. per hour per million cells for the first observation (at 15 minutes). The rate of respiration declined sharply for two hours, then remained fairly constant at about 3.8 cu. mm. O_2 per hour for several hours. In stationary-phase cultures (i.e. "old" cells), on the other hand, the initial average rate of oxygen consumption was 4 cu. mm. per hour per million cells; the rate declined gradually for two hours, then re-

mained fairly constant at a value of 1.9 cu. mm. O_2 per hour for several hours. Respiration rates differed somewhat even in cultures of the same age and growth phase.

Oxygen consumption in inorganic buffer was found to be approximately the same at hydrogen-ion concentrations between pH 4.5 and pH 7.9. (Results of typical experiments are shown in Table 1.)

TABLE 1

Effect of pH on respiration

(Duration of experiments three hours; M/40 sodium acetate used)

Exp. no.	Growth phase of cells	Oxygen consumption as cu. mm. O_2/hour/million cells					
		pH 4.5 inorganic acetate		pH 5.8 inorganic acetate		pH 7.9 inorganic acetate	
1	Logarithmic	4.59		4.91		5.20	
2	Stationary	2.49		2.43		2.48	
3	Stationary		5.75*	2.63	31.39		
4	Stationary			2.24	28.09		29.28

* Much cell injury.

Several experiments were performed to determine the respiratory quotient in inorganic buffer at pH 5.8. Most of the determinations of rate of carbon dioxide production were carried out without the customary addition of acid (see Dixon, 1934), since it was found that there was no appreciable accumulation of retained carbon dioxide in the cell suspensions under the conditions of these experiments. The respiratory quotient was usually found to be slightly less than one. (R. Q. values obtained in four three-hour experiments: 0.86, 0.92, 0.95, 1.04.)

II. *Effects of Various Organic Compounds*

In order to throw light on the nature of the substances and reactions involved in the respiratory metabolism of *Astasia,* the effect of various organic compounds on oxygen consumption was studied. Most of the substances tested serve as substrates for many types of cells; others are known to play some accessory part in certain cellular respiration systems. (See Needham and Green, 1938, for a recent account of the role of these compounds in cellular respiration.)

Usually 0.18 cc. of a M/2 test solution was added to 1.62 cc. of cell suspension in a Warburg vessel, and the respiration compared to that of control vessels containing cells suspended in inorganic buffer alone. Cultures of various ages were employed in these experiments. In most cases cultures in the stationary phase of growth were used. In each

separate experiment, oxygen consumption in the presence of the tested compound was expressed in terms of percentage of the control respiration. Results are shown in Table 2.

A definite increase in oxygen consumption was produced by the following: sodium formate, sodium acetate, sodium propionate, and ethyl alcohol (all M/20 concentrations), and M/40 sodium hexosediphosphate. Acetate had the most marked effect, with propionate and ethyl alcohol producing somewhat smaller increases. Rather slight but consistent in-

TABLE 2

Effect of various organic compounds on respiration

(M/20 concentrations used unless stated otherwise. Nearly all experiments of two hours' duration.)

Substances tested	Respiration as per cent of that in inorganic medium in separate experiments	Respiration as per cent of that in inorganic medium (average)
Sodium formate	192; 147; 151; 137	157
Sodium acetate	488; 731; 1130	783
Sodium propionate	428; 328; 229	328
Sodium butyrate	492;* 174;* 46;* 66*	195*
Sodium hexosediphosphate (M/40)	172; 163	168
Ethyl alcohol	833; 355; 278	489
Sodium citrate	115; 68; 99	94
Sodium beta-glycerophosphate	120; 86; 96	101
Sodium lactate	111; 113; 106	110
Sodium succinate	118; 96; 103	106
Galactose	132; 100; 110	114
Levulose	102; 113; 84	100
Glycerol	110; 127; 105	114
Dextrose	98; 107; 104	103
l-Xylose	96; 103; 129	109
Methyl alcohol	99; 127	113
Ethylene glycol	102	102
Potassium malonate (M/500)	100	100
Potassium fumarate (M/500)	99	99

* Much cell injury.

creases in respiration occurred in the presence of formate and hexosediphosphate. Inconsistent results were obtained with M/20 sodium butyrate; in some experiments it produced an increase in respiration, in other cases a decrease. Butyrate always caused injury or death in from 50 per cent to 90 per cent of the total cells present; the greater degree of injury was correlated with lower oxygen consumption. Various common substrates which are oxidized by many types of cells, such as simple sugars, lactate, succinate, etc., had no appreciable effect on the respiration of *Astasia*.

Since acetate produced the greatest increase in oxygen uptake, and since it has been shown to greatly increase growth of plantlike flagellates in general (Hall, 1941) and of *Astasia* in particular (Von Dach, 1940), the effects of this substance on respiration were studied in some detail.

Respiration in Acetate

At pH 5.8, when a solution of sodium acetate was tipped from the side-arm of a Warburg vessel into the main compartment containing

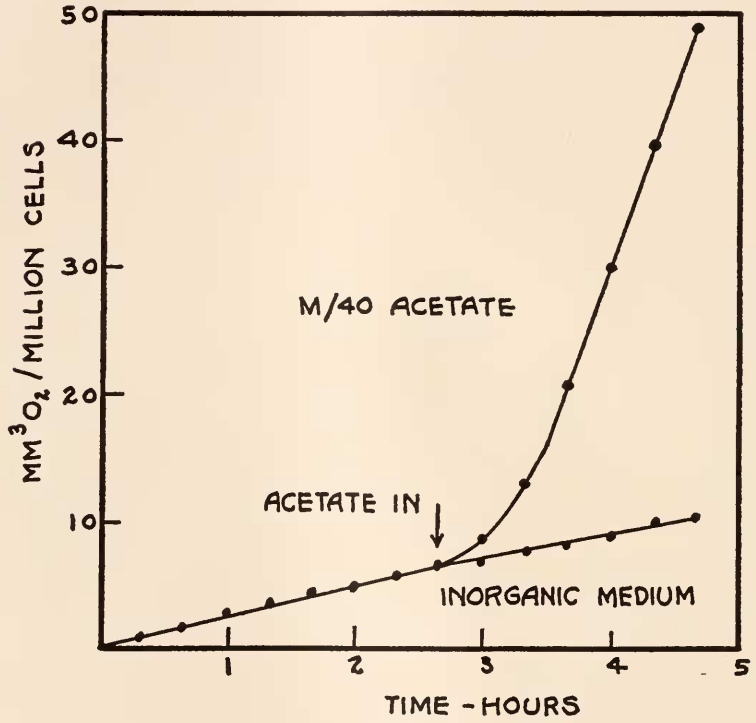

FIG. 2. Effect of adding acetate to cells in inorganic medium at pH 5.8. Results of a typical experiment. First 160 minutes: curve represents average respiration in four vessels containing cells in inorganic medium. M/40 acetate was added to two of the vessels at 160 minutes, at the arrow mark. From 160 to 280 minutes: upper curve represents average respiration in the two vessels to which acetate was added, lower curve represents average respiration in the two control vessels to which nothing was added.

cells in inorganic buffer, the rate of respiration rose steadily during a period of about one hour. (See Figure 2.) Thereafter respiration usually continued at a constant maximal rate for several hours. The acetate respiration data in this section deals with constant maximal rates.

Oxygen consumption in acetate was approximately the same at pH 5.8 and pH 7.9; but at pH 4.5 respiration was much less, and there was marked cell injury (Table 1). This differs from respiration in buffer alone, where oxygen consumption was approximately the same over the range pH 4.5–pH 7.9.

At pH 5.8 oxygen consumption was for a time approximately the same at acetate concentrations between M/20 and M/80 (see typical results in Table 3). However, after some time respiration in M/80 acetate declined rapidly, while that in M/20 and M/40 remained constant. It may be presumed that the enzyme system concerned was at first "saturated" with substrate at all these concentrations but as acetate was used up, the concentration in the M/80 vessels fell below the critical concentration for enzyme saturation.

TABLE 3

Effect of acetate concentration on oxygen consumption of logarithmic-phase cells
(Initial pH 5.8)

Time period	Control (inorganic buffer)	M/10 acetate	M/20 acetate	M/40 acetate	M/80 acetate
	cu. mm. O_2/hour/million cells				
0 to 60 min.....	5.72	39.28	55.50	62.36	64.27
60 to 120 min.....	4.42	44.46	61.40	67.52	69.12
120 to 180 min.....	4.01	46.96	64.88	68.39	19.23
180 to 240 min.....	3.37	48.62	66.58	69.56	4.80

Respiration in M/10 acetate was always markedly lower than in more dilute acetate concentrations (Table 3), although cell injury occurred in only one out of five experiments.

The growth phase of cells had a definite effect on their respiration in acetate. In numerous experiments it was found that in acetate medium, logarithmic-phase cells consumed from 40 to 60 cu. mm. O_2 per hour per million cells; while stationary-phase cells usually consumed from 20 to 30 cu. mm. O_2 per hour per million cells.

Experiments were performed to determine the respiratory quotient in acetate. In the CO_2 determinations bound carbon dioxide was liberated from the cell suspensions by tipping in 5N H_2SO_4 from the sidearms of the vessels (Dixon, 1934). The respiratory quotient in acetate was found to be approximately 1. (R. Q. values obtained in two experiments: 1.04, 1.03.) This value corresponds to the reaction for the complete oxidation of acetic acid to carbon dioxide and water:

$$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O.$$

The pH of cell suspensions in acetate usually increased somewhat (0.5 to 1.0 pH unit) during the course of an experiment.

No chemical tests were made to determine whether acetate was actually used up in the cell suspensions during these experiments. However, chemical analyses of old culture media have shown that the acetate concentration in cultures decreases with time (unpublished data). Hence it is reasonable to presume that the increase in respiration produced by acetate is due to the utilization and oxidation of this substance by the cells, rather than to some catalytic stimulating effect.

III. *Tests for Cytochrome*

The distribution of cytochrome among the Protozoa has received little attention; only a few records of its occurrence in members of this phylum have been published (cited by Jahn, 1941; Baker and Baumberger, 1941).

To determine whether cytochrome was present in *Astasia klebsii,* the usual method of spectroscopic examination was used. A very concentrated suspension of cells in acetate medium was placed in a small glass vial and examined by means of a Leitz micro-spectroscope. Three absorption bands were seen, located at approximately 605 mμ, 565 mμ, and 555 mμ; these values agree fairly well with the generally reported locations of bands for cytochromes a, b, and c respectively (see Oppenheimer and Stern, 1939). Further evidence that these absorption bands were caused by cytochromes was furnished by the following observations:

(1) The bands disappeared after vigorous aeration of the suspension, then gradually reappeared and regained full intensity. (Aeration converts reduced cytochromes to oxidized cytochromes, which return to the reduced form when reducing conditions again prevail.)

(2) Addition of M/100 NaCN to the cell suspension did not change the absorption spectrum, but did prevent removal of the absorption bands by aeration. (Cyanide inhibits cytochrome oxidase, so that the cytochromes perforce remain in the reduced form.)

The oxidation of para-phenylene-diamine (abbreviated as PPD) has often been used as a test for the cytochrome-cytochrome oxidase complex, although this test is not wholly specific nor unambiguous (see Stotz, 1939, and Hogness, 1939). The effect of various concentrations of PPD upon oxygen consumption of *Astasia* was tested in several experiments. In a single instance M/200 PPD produced a 70 per cent increase in respiration and did not injure the cells; in all other experi-

ments, at PPD concentrations between M/20 and M/1000, the respiration was equal to or below the control respiration and nearly always extensive cell injury occurred.

IV. *Effects of Respiratory Inhibitors*

A. Cyanide

Cyanide is generally regarded as a useful tool for determining how much of the respiration of any organism is carried on through the cytochrome-cytochrome oxidase system, and has so been used in studies on numerous types of cells and tissues (review by Commoner, 1940). The spectroscopic detection of cytochrome in *Astasia klebsii* made it seem

TABLE 4

Effect of M/100 NaCN on the increase in respiration produced by various substrates at pH 5.8

Substrate tested	Exp. 1 Ethyl alcohol (M/20)	Exp. 2 Sodium formate (M/20)	Exp. 3 Sodium acetate (M/40)	Exp. 4 Sodium propionate (M/20)	Exp. 5 Sodium hexosediphosphate (M/40)
	cu. mm. O_2/hour/million cells				
Respiration in inorganic buffer before adding substrate (control)	2.93	5.73	5.40	5.73	5.71
Respiration after adding substrate	8.15	8.45	41.43	18.81	8.41
(% of control)	(278%)	(147%)	(767%)	(328%)	(163%)
Respiration after adding M/100 cyanide to cell suspension in substrate	3.31	3.35	1.93	3.60	5.37
(% of control)	(113%)	(59%)	(36%)	(63%)	(94%)

likely that the cytochrome-cytochrome oxidase system played an important part in its respiration. Experiments on the effect of cyanide were accordingly undertaken.

Preliminary experiments were performed to determine the effect of cyanide on respiration in solutions of those substrates which had previously been found to increase oxygen consumption. It was found that the increase in respiration rate produced by formate, acetate, propionate, hexosediphosphate, and ethyl alcohol was usually completely abolished by M/100 sodium cyanide (Table 4).

Subsequently more detailed experiments on cyanide effect on respiration in inorganic buffer and in acetate medium were performed. The

cyanide solutions used were prepared by diluting M/1 NaCN with inorganic buffer of the same pH as the cell suspensions. The cyanide-KOH mixtures described by Krebs (1935) were employed as carbon dioxide-absorbing fluids, to prevent the escape of HCN from the cell suspensions during the course of an experiment.

Cyanide inhibition of respiration, both in inorganic buffer and in acetate, was found to be approximately the same at pH 5.8 and pH 7.9.

Cyanide inhibition of respiration is essentially a reversible reaction. It was necessary to show that cyanide did not visibly damage the cells, and that respiration went back to normal after removing the cyanide, before concluding that the only effect of cyanide was inactivation of the cytochrome-cytochrome oxidase system. Experiments along these lines were performed, employing cyanide solutions of various concentrations.

TABLE 5

Recovery of respiration from cyanide inhibition
(Logarithmic-phase cells in inorganic buffer, pH 5.8)

NaCN concentration	Respiration in cyanide (2 hours)	Recovery respiration (after removal of cyanide by washing) (2 hours)
	cu. mm. O_2/hour/million cells	
0	7.76	3.52
M/250	5.11	7.08
M/100	3.40	7.61
M/50	2.39*	5.20*

* Slight cell injury.

Using the erythrosin test, it was found that M/20 NaCN injured all astasias present within two hours. M/50 concentration usually injured a small percentage of the cells present. In M/100 cyanide there was scarcely any cell injury after four hours.

"Recovery" experiments were performed as follows: After a two-hour run in cyanide, the cell suspension in each Warburg vessel was removed and separately washed four times by centrifuging in inorganic buffer to remove the cyanide, then returned to the washed Warburg vessel for further observations on respiration. Results of a typical recovery experiment are shown in Table 5. These results show that when cells which had been in M/100 and M/250 cyanide were washed cyanide-free their rate of respiration rose to approximately the initial control level; for cells which had been in M/50 cyanide the recovery rate was somewhat below the initial control. Since M/50 cyanide proved somewhat toxic, it was not used in later experiments.

Figure 3 shows the results of a number of experiments on the effect of various concentrations of cyanide on respiration in inorganic buffer and in acetate, for logarithmic-phase cells and for stationary-phase cells. The experiments were performed at pH 5.8, using cyanide concentrations between M/100 and M/500,000.

For stationary-phase astasias under maximum cyanide inhibition (in M/100 cyanide), it was found that the cyanide-stable respiration was practically the same whether substrate was present or absent. In these

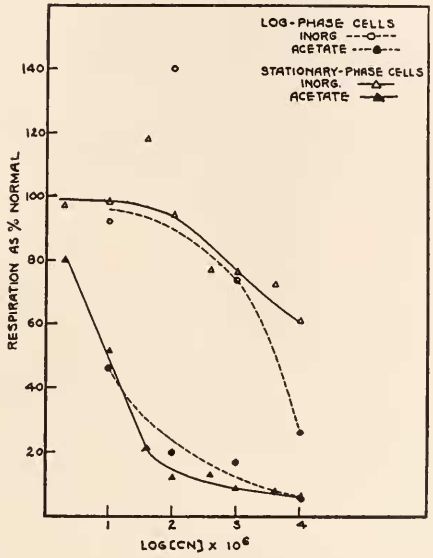

FIG. 3. Effect of different cyanide concentrations on respiration. Average values from three logarithmic-phase experiments and four stationary-phase experiments are represented (two-hour experiments at pH 5.8).

Normal respiration (average) as cu. mm. O_2/hour/million cells:

Log-phase cells in inorganic medium, 6.30
Log-phase cells in M/40 acetate, 46.96
Stationary-phase cells in inorganic medium, 2.28
Stationary-phase cells in M/40 acetate, 24.63

experiments, the average cyanide-stable respiration (expressed as cu. mm. O_2/hour/million cells) was 1.37 in inorganic buffer and 1.46 in acetate. In inorganic medium the total (cyanide-free) respiration was small, so that the percentage of inhibition by M/100 cyanide was relatively slight. But when substrate in sufficient concentration (M/40 sodium acetate) was present the cyanide-sensitive system presumably functioned at full capacity, a high total (cyanide-free) respiration was attained, of which the cyanide-stable portion formed only a small frac-

tion—consequently over 90 per cent of the respiration of substrate-saturated cells was cyanide-sensitive. For logarithmic-phase cells in M/100 cyanide the situation was much the same. The foregoing is in agreement with Commoner's (1940) statement: "Since most variations in the total rate of respiration are mainly due to variations in the activity of the cyanide-sensitive system alone, percentage inhibition by cyanide (i.e. "cyanide-sensitivity") increases with the normal rate of respiration."

Cyanide at high concentration (M/100 and M/1000) produced a marked decrease in the amount of paramylum in the cells in long-term experiments. It seems possible that this phenomenon may have been due to the effect of cyanide in increasing activity of amylase, thus resulting in more rapid breakdown of paramylum. The effect of cyanide in stimulating amylase activity under certain conditions has been demonstrated by several workers (Hanes and Barker, 1931; Denny, 1931).

B. Azide

Keilin (1936) studied the inhibition of cellular respiration by sodium azide (NaN_3), and showed that its mode of action was in general similar to that of cyanide. However, he and later workers pointed out certain

TABLE 6

Effect of pH on azide inhibition of respiration of stationary-phase cells

NaN_3 concentration	At pH 5.8		At pH 7.9	
	Inorganic buffer	M/20 acetate	Inorganic buffer	M/20 acetate
	cu. mm. O_2/hour/10^6 cells		*cu. mm. O_2/hour/10^6 cells*	
0 (Control)	1.92	26.51	1.96	26.52
M/1000	2.90		2.31	
M/500		1.26		26.23
Azide inhibition	(Up 51%)	(95%)	(Up 18%)	(1%)

(No cell injury occurred in this experiment.)

interesting differences in the effects of these two substances. In view of these facts a study of azide effects was included in the present investigation.

The relation between pH and azide inhibition of respiration in *Astasia* was investigated (Table 6). In acetate medium, M/500 azide had no effect at pH 7.9, but produced 95 per cent inhibition of respiration at pH 5.8. In inorganic buffer there was no azide inhibition at either pH level.

Somewhat similar relations between pH and azide inhibition have been reported by Keilin (1936), who studied the effect of azide on respiration of yeast cells, and by Armstrong and Fisher (1940), who studied the effect of azide on frequency of the embryonic fish heart. Pointing out that at low pH much of the azide would be in the form of

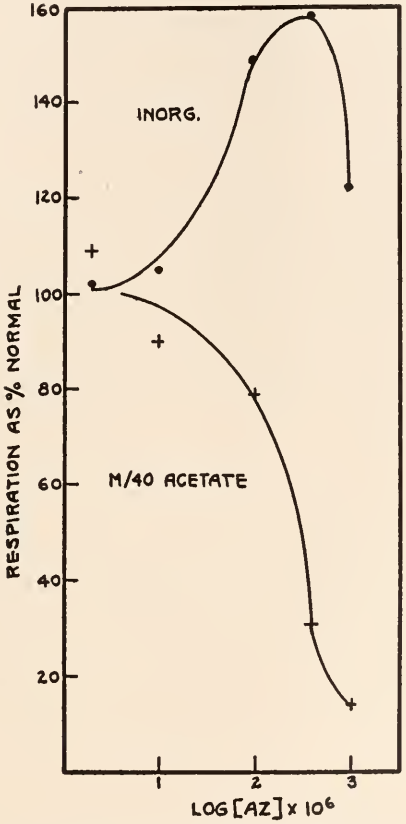

FIG. 4. Effect of different azide concentrations on respiration at pH 5.8. Average values from six two-hour experiments, using cells in logarithmic and stationary phases of growth.

undissociated hydrazoic acid, Armstrong and Fisher concluded that only in this undissociated form could azide enter fish embryo cells. On the other hand, Stannard (1939) found no such pH effect involved in azide inhibition of respiration of active frog muscle.

Experiments were performed at pH 5.8 to determine the toxic level of azide concentration. It was found that in M/250 azide nearly all the cells were injured within two hours (five out of five experiments).

M/500 azide usually injured a large percentage of the cells present (seven out of eight experiments). In M/1000 azide no cell injury was observed in most cases (10 out of 12 experiments). Azide was thus toxic to the cells at much lower concentrations than cyanide.

The effect of azide concentrations between M/500,000 and M/1000 on respiration at pH 5.8 was investigated. Results are shown in Figure 4. In acetate medium, an inhibition curve more or less resembling that produced by cyanide was obtained. But in inorganic buffer, no azide inhibition occurred at any concentration tested, while at certain azide concentrations there was a pronounced increase in respiration over the control values.

Stannard (1939) suggested that in frog muscle there appear to be two distinct respiratory mechanisms: one is characteristic of resting muscle and is only cyanide sensitive; the other functions only in activity, includes the cytochrome system, and is both azide and cyanide sensitive. Similarly, in *Astasia,* it appears that respiration in inorganic medium is mediated through a cyanide sensitive but azide insensitive system; while respiration in acetate takes place through a system which is both azide and cyanide sensitive.

Summary

1. Concentrated cell suspensions of *Astasia klebsii* were prepared from pure cultures. The respiration of these suspensions was measured by the Warburg direct method at 25.2° C., usually at pH 5.8.

2. Cells in inorganic medium showed an early period of declining respiration, followed by an approximately constant period. During the constant period, old (stationary-phase) cells consumed about 1.9 cu. mm. O_2/hour/million cells, and young (logarithmic-phase) cells consumed about 3.8 cu. mm. O_2/hour/million cells.

3. The effects of numerous organic compounds on respiration were tested. Only formate, acetate, propionate, ethyl alcohol, and hexosediphosphate produced a definite increase in oxygen consumption, the greatest increase occurring in acetate.

4. In inorganic medium, respiration was approximately the same between pH 4.5 and pH 7.9. In acetate, respiration was the same at pH 5.8 and pH 7.9, and much lower at pH 4.5.

5. In acetate the respiratory quotient was 1.0; in inorganic medium it was slightly less than 1.0.

6. The presence of cytochrome in the cells was detected by spectroscopic examination.

7. The highest non-toxic concentration of cyanide (M/100) produced about 95 per cent inhibition of respiration in acetate, and a lesser degree of inhibition in inorganic medium. The highest non-toxic concentration of azide (M/1000) inhibited nearly 90 per cent of the respiration in acetate, but had no inhibitory effect on respiration in inorganic medium.

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